when dried, and wherein the surface active agent comprises a sugar in a hydrophilic part of the surface active agent.

Applicants' claim 12 recites a method for manufacturing a chromatography medium which comprises a reactive layer on which at least one reactive component for a chromatographic analysis is immobilized comprising: (a) impregnating or coating the reactive layer of the chromatography medium with a liquid, wherein a surface active agent which is solidified when dried is dissolved in the liquid; and (b) drying the reactive layer which has been impregnated or coated with the liquid in which the surface active agent is dissolved, wherein the surface active agent comprises a sugar in a hydrophilic part of the surface active agent.

Applicants' invention results in reduction of the influences to the reactive component (specific protein) which is immobilized to the reactive layer, and reduction of the denaturation or deactivation of the reactive component to the minimum. Therefore, the preservation stability of the chromatography medium is enhanced, the quality preservation period is lengthened and relaxation of the maintenance condition is made possible.

There are three main causes which deteriorate the preservation performance of a chromatography medium in the analysis of a liquid sample.

(1) Deterioration of the permeation property of the reaction layer of the chromatography medium, and reduction of the liquid flow amount compared to the initial stage.

(2) Deterioration of the solvability of the marker reagent which is impregnated, and reduction of the amount of the marker reagent relating to the reaction compared to the initial stage.

(3) Deactivation of the marker reagent or the specific protein immobilized on the reaction layer.

The above three causes result in a reduction in detection sensitivity, such as from 10 to 2 or 5, or a deterioration in precision, such as from CV5% to CV20%. The object of the present invention is to provide a chromatography medium which can solve these problems, and which can maintain the performance of the chromatography medium at high sensitivity and at high precision, even when maintained for a long period of time. For example, a chromatography medium which had a detection sensitivity of 10 at an initial state, and which maintains this detection sensitivity at a state unlimitedly close to 10, for a long period of

time, as well as keeping its measurement precision which was CV5% at an initial state, at a state unlimitedly close to 5%, for a long period of time.

Since the reactive layer which is used in a chromatography medium has a behavior of developing a liquid sample, in an analysis of a liquid sample utilizing a chromatography medium, the maintenance of the permeability of the reactive layer is quite important. A method of carrying out a surface active agent processing is often employed to avoid the deterioration of the permeability of the reactive layer, as discussed in (1) above. By enhancing the permeability of this reactive layer in the surface active agent processing, the reactive layer is able to carry out development of a sufficient amount of liquid sample to the downstream region of a chromatography medium, even in a chromatography medium which is maintained for a long period of time.

Absorption of the marker reagent into the reactive layer or the impregnating filter paper in the maintaining period is a major cause of the deterioration of the solvability of the marker reagent, as discussed in (2) above. In order to solve this problem, a method of carrying out surface active agent processing on the impregnating part, or mixing the surface active agent into the marker reagent is adopted. This method allows solvability by suppressing the remaining amount of the marker reagent to the minimum, even with keeping the permeability of the reaction layer even after a long period. However, the surface active agent, which is used to improve problems (1) and (2) discussed above, causes deactivation of the marker reagent or the protein which is immobilized onto the reactive layer.

The reactive component (protein) has a stereo-type structure which is made of fine strings, and it is in a state where the stereo-type structure is likely to be destroyed. Specifically, when the reactive component (protein) is in solution, it is likely to be denatured or deactivated, because the bindings which support the stereo-type structure are likely to be separated from each other. On the other hand, the stereo-type structure is not likely to be destroyed in its dry state. Therefore, the preservation stability of the reactive component is naturally superior in its dry state compared to its solution state.

The surface active agent is likely to unfavorably affect proteins. If a surface active agent which is not likely to become a dry state and holds a little amount of water therein is employed, or one which is likely to absorb moisture from the environment is employed,

then both the reactive layer and the reactive component (protein) which is immobilized on the reactive layer are also in a state including water. Therefore, the reactive component is likely to be denatured or deactivated, and therefore will not stand a long period of maintenance. However, if the surface active agent is in a dry state, the reactive layer and the reactive component will also be in a dry state, and therefore it will be possible to maintain a long term preservation performance.

However, it is still very difficult to maintain the preservation performance for a long period of time, even in a dry state. In the food business, it is widely known to employ sugar as a preservation agent in order to avoid denaturation of proteins. However, even if the denaturation of deactivation of the protein is prevented, the solubility of sugar would result in deterioration of the permeability of the reaction layer.

The present invention provides a surface active agent which can be in a dry state and has a sugar in its hydrophilic part, which is used to process the reactive layer, thereby preserving the permeability of the reactive layer for a long period of time as well as protecting the specific proteins by the function of sugar. Further, the surface active agent of the present invention is one which can become solid when dried, and which preserves the performance of the specific proteins by making the same in a dry state which includes no water and does not become a wet state by a drying processing, thereby enabling the enhancement of the preservation stability of the chromatography medium, and lengthening of quality preservation period and the relaxation of the maintenance condition.

Arguments regarding Chu in view of Nanbu et al.

Chu relates to a method of assembling an analytical device that is used in the detection of a target substance in a liquid sample, and an assay using the analytical device. The invention of Chu comprises a surfactant-treated porous reaction membrane provided with at least one receptor area, which is in a limited region of the surfactant-treated porous reaction membrane. Chu teaches that the at least one receptor area has a higher concentration of surfactant than the areas of the sample-contacting surface that are peripheral to the limited region. (See column 1, lines 49-51 of Chu.). Further, Chu teaches that the surfactant causes the liquid sample to flow faster through the portions of the reaction membrane where receptor molecules are located. Therefore, since a higher concentration of surfactant is present in the receptor area in the porous

reaction membrane, the liquid sample passes through this receptor area faster than through the periphery, thereby increasing the assay sensitivity. (See column 1, lines 60-64 of the specification.)

In other words, in the Chu patent, the enhancement of assay sensitivity at a reaction area of the reaction membrane is intended, and the reaction area is processed to include a higher concentration of surfactants than the other areas in the reaction membrane. This results in the liquid sample passing through the reaction area rapidly, so that the significant quantity of liquid sample can pass through the reaction area compared to its periphery, resulting in an increase of the assay sensitivity. Chu fails to teach or suggest enhancing the permeability of the reaction layer, maintaining long-term permeability of the reactive layer, processing the entire reaction layer with the surface active agent, or employing a particular surface active agent which is solidified when dried and comprises sugar in a hydrophilic part to prevent denaturing or deactivation of the reactive component (protein).

The method of Chu does not teach or suggest processing the entire reaction layer with the surface active agent, in order to enhance the permeability of the entire reaction layer. On the contrary, Chu teaches reacting a particular area (the reaction area) to include a higher concentration of surfactant than the peripheral areas, thus teaching away from Applicants' claimed invention. Furthermore, Chu fails to teach or suggest employing a suface active agent which comprises sugar in a hydrophilic part , in order to prevent deactivation of the reactive component.

The teachings of Nanbu et al. fail to remedy the deficiencies of Chu.

Nanbu et al. disclose a method for measuring the concentration or the activity of UTI, wherein a urine sample, a buffer solution, a trypsin solution and a substrate solution are mixed and the trypsin activity is then measured, and wherein a surface active agent is included in either the buffer solution or the enzyme solution. Nanbu et al. further disclose that the use of the surfactant improves the protease activity, thereby improving the measurement sensitivity. (See column 2, lines 46-48 of Nanbu et al.) The reference also discloses that the UTI sensitivity is improved when using the surfactant mixed in the buffer solution, and that when the concentration of the surface active agent is increased, the UTI measurement sensitivity is also enhanced. (See column 7, lines 58-62 of Nanbu et al.)

First, as discussed above, Chu teaches processing a particular area with a high

concentration of surfactants, rather than processing the entire reaction layer. Nanbu et al. do not teach or suggest altering the teachings of Chu to process the entire reaction layer with the surface active agent.

Second, Chu fails to teach or suggest using a surface active agent which comprises sugar in a hydrophilic part. Although Nanbu et al. teach a surface active agent having a sugar in its hydrophilic part as an example of surface active agent which enhances the activity of the protease, most of the examples of surface active agents which enhance the activity of protease are those which do not have sugar in their hydrophilic part. Nanbu et al. do not disclose or suggest choosing a surface active agent which has sugar in its hydrophilic part in order to enhance the activity of the enzyme, or that the activity of the enzyme is enhanced by the function of the sugar in the hydrophilic part.

As stated by the Examiner, Applicants previously set forth arguments that the Examiner did not provide a reason why one of ordinary skill in the art would select a surface active agent that is solidified when dried and comprises a sugar in a hydrophilic part. The Examiner states (in the middle of page 8 of the Office Action) that this argument is not found persuasive because the Examiner has taken notice of the equivalence of sucrose monolaurate (which contains a sugar in a hydrophilic part) to polyoxyethylene sorbitan monolaurate and polyoxyethylene sorbitan monooleate (which are taught in Chu). However, this response does not address Applicants' question as to why one of ordinary skill in the art would even choose sucrose monolaurate from the many surfactants taught by Nanbu et al. Even if the Examiner's assertion of equivalency is accurate, the Examiner has provided no reason why one of ordinary skill in the art would select this particular surfactant from the list provided in Nanbu et al. Further, the Examiner has provided no reason why one of ordinary skill in the art would select a surface active agent that is solidified when dried and comprises a sugar in hydrophilic part from the many surfactants disclosed by Nanbu et al. The Examiner asserts that he has taken notice of the equivalence of sucrose monolaurate to the surfactants of Chu. However, without a suggestion or motivation to select sucrose monolaurate from the list of surfactants in Nanbu et al., any equivalence between the surfactants is irrelevant.

Third, Nanbu et al. disclose that surface active agent is employed in order to enhance

the activity of the enzyme, and that the measurement sensitivity is enhanced by enhancing the activity of the enzyme. In other words, in the invention of Nanbu et al., the detection sensitivity which was 10 in the prior art is enhanced to a value such as 20 or 30. On the contrary, in Applicants' invention, the surface active agent is employed to enhance the permeability of the reaction layer, to maintain the permeability for a long period of time, and to prevent the denaturation or deactivation of reactive component (protein) by the function of the sugar in the hydrophilic part in long-term preservation thereby maintaining the performance of the specific protein. Specifically, Applicants' invention results in maintaining the detection sensitivity which is 10 at an initial state at a state unlimitedly close to 10 for a long period of time. Applicants' invention does not enhance the reaction activity of the specific protein.

Nanbu et al. do not teach or suggest employing a surface active agent in order to prevent the denaturation or deactivation of the enzyme, to enhance the preservation stability of the reagent solution, and to maintain the detection sensitivity.

Fourth, the Examiner asserts that one of ordinary skill in the art would have a reasonable expectation of success using the surface active agent (surfactants) of Nanbu et al. which is an equivalent of the surface active agent of Chu in place of the surface active agent of Chu.

However, if a surfactant such as n-octyl-β-D-thioglucoside or sucrose monolaurate, as taught in Nanbu et al., is employed in Chu, since the effect of these surfactants taught in Nanbu et al. is enhancement of the activity of the enzyme, the effect which is presumed when Nanbu et al. is combined with Chu is an enhancement of assay sensitivity of the receptor area on the reaction membrane. Specifically, the activation of the reaction between the target substance and the receptor area, i.e., the antigen-antibody reaction. Particularly, when Chu and Nanbu et al. are combined, one of ordinary skill in the art would expect enhancement of the reaction activity of the specific protein, or enhancement of the detection sensitivity, i.e., enhancing the detection sensitivity to a value such as 20 or 30 which is higher than 10.

There is no suggestion of the expectation of maintaining long-term permeation, or prevention of denaturation or deactivation of specific protein by the function of sugar in the hydrophilic part in long term preservation thereby maintaining the performance of the

specific proteins, i.e., preserving the detection sensitivity which was 10 at an initial state in a state unlimitedly close to 10 for a long period of time.

Accordingly, the present invention which reduces the influences to the specific proteins which are immobilized to the reaction layer by using a surface active agent having sugar in a hydrophilic part and being able to become solid when dried is a new immuno-chromatography apparatus invented by the present inventors. Applicants' invention results in the following advantages: suppression of the denaturation or deactivation of the specific proteins to the minimum, and enhancement of the preservation ability, lengthening of the quality preservation period, and relaxation of the maintenance condition of the chromatography medium.

In summary, Applicants' claimed invention is patentable over the combination of Chu and Nanbu et al. for the following reasons:

1. Chu teaches processing a particular part of the reactive layer to contain a higher concentration of surfactants than the other areas, in order to increase assay sensitivity. Applicants' invention processes the entire reactive layer, rather than a portion of the reactive layer, with a surface active agent. The teachings of Nanbu et al. fail to remedy this deficiency.

2. Chu fails to teach or suggest a surface active agent which is solidified when dried and comprises a sugar in its hydrophilic part. Nanbu et al. disclose a long list of surfactants, which includes sucrose monolaurate. However, there is no motivation to choose this particular surfactant from the many disclosed by Nanbu et al. Therefore, whether this surfactant is equivalent to those taught by Chu is irrelevant.

3. Nanbu et al. teach improving measurement sensitivity, while Applicants' invention maintains measurement sensitivity.

4. The combination of references does not teach or suggest a chromatography medium which maintains long-term permeation, and which prevents the denaturation or deactivation of a specific protein by the function of sugar in the hydrophilic part for long term preservation, thereby maintaining the performance of the specific protein.

Arguments regarding Chu in view of Uenoyama et al.

Uenoyama et al. provide a method of measuring the in-urine protease obstruction substance by mixing a urine sample, a protease solution, and buffer solution, adding a substrate solution thereto to cause the enzyme reaction, and measuring the activity of the enzyme. The adjustment of the substrate solution is carried out by solving the substrate in an organic solvent, and adding at least one surface active agent, chosen from either an amphoteric surfactant or a nonionic surfactant, into a solution comprising at least one of an organic solvent and an aqueous medium.

This method employs a surface active agent in order to solve the slightly soluble substrate which is soluble by an organic solvent by using the organic solvent which is likely to inhibit the activity of the enzyme at minimum that is required, and in order to not precipitate the substrate. Further, the surface active agent is used so that the amount of an organic solvent can be reduced, and a slightly soluble substrate can be used in a sufficient amount. (See column 3, lines 21-24 of Uenoyama et al.). Additionally, because the substrate can be used in a sufficient amount, precision and reproducibility of the measurement can be improved. Further, by the use of the specific surfactants, the solubility of the substrate is also improved, so that crystallization of the substrate can be prevented. (See column 3, lines 24-31 of Uenoyama et al.) It is also described that if the nonionic surfactants is not used, the substrate crystallizes. (See column 9, lines 28-31 of Uenoyama et al.)

In Uenoyama et al., by using the surface active agent in order to enhance the solubility of the substrate, the amount of organic solvent which inhibits the activity of the enzyme by denaturing or deactivating the enzyme can be reduced, and as a result, the denaturation or deactivation of enzyme is suppressed and the activity and measurement sensitivity of the enzyme is enhanced. Although Uenoyama et al. teach a surface active agent which has sugar in its hydrophilic part as an example, most of the examples of surface active agents are those which do not have sugar in their hydrophilic part. It is clear that the measurement sensitivity is not necessarily enhanced by the function of the surface active agent having sugar in its hydrophilic part. Further, the role of the surface active agent in Uenoyama et al. is the enhancement of solubility of the slightly soluble substrate and the reduction of use amount of organic solvent.

The surface active agent in the present invention is intended to enhance the permeability of the reaction layer as well as to maintain the permeability for a long period of time, to preserve the performance of the specific protein by preventing the denaturation or deactivation of the specific protein by the function of sugar in the hydrophilic part in the long-term preservation. Applicants' invention does not suggest that the reaction activity of the specific protein is enhanced, or that the detection sensitivity which was 10 in the prior art is enhanced to a higher value. On the contrary, even if Uenoyama et al. is considered to suggest that the surface active agent functions indirectly as preventing the denaturation or deactivation of enzyme, it is absolutely an indirect function, and it is not directly contributing to the enhancement of the enzyme activity. Also, there is neither disclosure nor suggestion that the surface active agent is employed as the reagent enhancing preservation stability in order to preserve the reagent for the long term, or that the surface active agent enhances the preservation stability of the enzyme solution by protecting the enzyme by sugar in the hydrophilic part.

The teachings of Uenoyama et al. do not remedy the deficiencies of Chu.

Initially, as discussed above, Chu teaches processing a particular area with a high concentration of surfactant, rather than processing the entire reaction layer. Uenoyama et al. do not teach or suggest altering the teachings of Chu to process the entire reaction layer with the surface active agent.

Second, Chu fails to teach or suggest a surface active agent comprising sugar in a hydrophilic part. Applicants previously set forth arguments that the Examiner has not provided a reason why one of ordinary skill in the art would select a surface active agent that is solidified when dried and comprises a sugar in a hydrophilic part from the 16 types of surfactants disclosed by Uenoyama et al. The Examiner states (at the top of page 10 of the Office Action) that n-octyl-B-D-thioglucoside and sucrose monolaurate are equivalent to the surface active agents taught by Chu. However, this response does not address Applicants question as to why one of ordinary skill in the art would even choose n-octyl-B-D-thioglucoside or sucrose monolaurate from the many surfactants taught by Uenoyama et al. Even if the Examiner's assertion of equivalency is accurate, the Examiner has provided no reason why one of ordinary skill in the art would select this particular surfactant from the many surfactants disclosed in

Uenoyama et al. Further, the Examiner has provided no reason why one of ordinary skill in the art would select a surface active agent that is solidified when dried and comprises a sugar in hydrophilic part from the many surfactants disclosed by Uenoyama et al. Although the Examiner asserts that n-octyl-B-D-thioglucoside or sucrose monolaurate are equivalent to the surfactants of Chu, without a suggestion or motivation to select n-octyl-B-D-thioglucoside or sucrose monolaurate from the list of surfactants in Uenoyama et al., any equivalence between the surfactants is irrelevant.

Third, the Examiner asserts that one skilled in the art would have expected success by employing a surface active agent of Uenoyama et al., which is an equivalent of the surface active agent of Chu, in place of the surface active agent of Chu.

The effect of these surface active agents taught in Uenoyama et al. is enhancement of solubility of a slightly soluble substrate, and thereby it is intended that the use amount of organic solvent is reduced and the activity of the enzyme is indirectly enhanced. However, there is no slightly soluble substrate or organic solvent used in Chu, and therefore there is no reason to employ a surface active agent which is intended to enhance the solubility of a slightly soluble substrate, as taught by Uenoyama et al.

Because one of ordinary skill in the art would not employ a component intended to enhance the solubility of a slightly soluble substrate when the device does not contain a slightly soluble substrate, it is impossible to predict a result. Further, it is impossible to expect long-term permeation preservation, or the preservation of the performance of the specific protein by preventing the denaturation and the deactivation of the specific protein by the function of sugar in the hydrophilic part in long-term preservation.

Accordingly, the present invention which reduces the influences to the specific proteins which are immobilized to the reaction layer by using the surface active agent having sugar in a hydrophilic part and being able to become solid when dried condition is a new immuno-chromatography apparatus invented by the present inventors. Applicants' invention results in the following advantages: suppression of the denaturation or deactivation to the minimum, enhancement of the preservation stability, lengthening of the quality preservation period, and relaxation of the maintenance.

In summary, Applicants' claimed invention is patentable over the combination of Chu and Uenoyama et al. for the following reasons:

1. Chu teaches processing a particular part of the reactive layer to contain a higher concentration of surfactants than the other areas, in order to increase assay sensitivity. Applicants' invention processes the entire reactive layer, rather than a portion of the reactive layer, with a surface active agent. The teachings of Uenoyama et al. fail to remedy this deficiency.

2. Chu fails to teach or suggest a surface active agent which is solidified when dried and comprises a sugar in its hydrophilic part. Uenoyama et al. disclose 16 types of surfactants, which include n-octyl-B-D-thioglucoside or sucrose monolaurate. However, there is no motivation to choose one of these particular surfactants from the many disclosed by Uenoyama et al. Therefore, whether these surfactants are equivalent to those taught by Chu is irrelevant.

3. Uenoyama et al. employ a surface active agent in order to enhance the solubility of a slightly soluble substrate. However, there is no slightly soluble substrate or organic solvent used in Chu, and therefore there is no reason to employ a surface active agent which is intended to enhance the solubility of a slightly soluble substrate, as taught by Uenoyama et al.

4. The combination of references does not teach or suggest that the surface active agent is employed as the reagent enhancing preservation stability in order to preserve the reagent for the long term, or that the surface active agent enhances the preservation stability of the enzyme solution by protecting the enzyme by sugar in the hydrophilic part.

For these reasons, the invention of claims 5, 12, 27, 31, 41, 45, 53 and 60 is clearly patentable over Chu in view of Nanbu et al. or Uenoyama et al.

The rejection of claim 49 under 35 U.S.C. § 103(a) as being unpatentable over Chu in view of Nanbu et al. or Uenoyama et al. and further in view of Iwata et al. is respectfully traversed.

The comments set forth above are equally applicable to this rejection. Since claim 49 is directly dependent on claim 12, the subject matter of claim 49 is patentable over Chu in view of Nanbu et al. or Uenoyama et al. for the same reasons that the subject matter of claim 12 is patentable over this combination of references. The teachings of Iwata et al. do not remedy the deficiencies of these references.

Conclusion

Therefore, in view of the above remarks, it is submitted that each of the grounds of rejection set forth by the Examiner has been overcome, and that the application is in condition for allowance. Such allowance is solicited.

Respectfully submitted,

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